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L12: Entry 4 of 6

File: USPT

DOCUMENT-IDENTIFIER: US 5853576 A
TITLE: Phytorecovery of metals using seedlings

US PATENT NO. (1):
5853576

Prior art?
No, because hydroponic media is used
claims air regum
the appl. regum
gum

Detailed Description Text (6):

Etiolated Brassica juncea seedlings were used to deplete the metal [cadmium (II), cobalt (II), copper (II), manganese (II), nickel (II), strontium (II), zinc (II), arsenic (II), chromium (II), and uranium (VI)] content of metal-containing aqueous solutions. Two hundred and fifty milligrams (mg) of B. juncea seeds previously stored in the dark were exposed to 800 milliliters (ml) of deionized water in a light-excluding 1 liter (l) plastic beaker (approximately pH 5.5) at 22.degree.-25.degree. C. The seed-containing aqueous composition was aerated using a sparging stone connected to a laboratory air pump, thereby mixing the composition. This process was repeated using similar materials on a daily basis for a total of 13 days. On every third day, the deionized water was exchanged for fresh deionized water. As a consequence, contained biomasses of progressively developed B. juncea seedlings were formed in 13 separate beakers. On day 14, seedlings at the various developmental stages were separately transferred to 13 large plastic tubs. Each tub contained 10 liters of a solution of metals as follows, with the final concentration of the relevant metal or metalloid in parentheses: NaAsO.₄ (0.5 mg/l As), Cd(NO₃)₂·2.4H₂O (0.1 mg/l Cd), K₂Cr₂O₇ (0.2 mg/l Cr), Co(NO₃)₂·6H₂O (0.5 mg/l Co), Cu(NO₃)₂·2·3H₂O (0.5 mg/l Cu), MnCl₂·2.4H₂O (3 mg/l Mn), Ni(NO₃)₂·2·6H₂O (0.2 mg/l Ni), NaSeO₃ (0.5 mg/l Se), SrCl₂·2.6H₂O (0.5 mg/l Sr), UO₂·2(C₂H₃O₂)₃ (0.5 mg/l U), and Zn(NO₃)₂·2.6H₂O (2 mg/l Zn). The pH of the solution of metals was approximately 5.0-5.5 prior to the addition of seedlings; the pH after incubation of the seedlings was approximately 5.0. Seedlings were incubated in the solution of metals with continuous aeration for 48 hours in the dark at a temperature of 22.degree.-25.degree. C. Each solution of metals was replaced with a fresh solution of metals after 24 hours.

Detailed Description Text (10):

One of skill in the art would recognize that the seedlings of the invention may vary in terms of the rate of metal accumulation, depending on a variety of factors. These factors include the ratio of soluble to insoluble metal in the metal-containing aqueous solution, the type of metal-containing aqueous solution, the total metal concentration, pH, organic matter content, and temperature.

Detailed Description Text (12):

In another embodiment of the methods of the invention, contained biomasses of a variety of plant species independently depleted metals in a metal-containing aqueous solution. In particular, B. napus (cv. unknown), B. napus (cv. Westar), B. rapa (cv. unknown), B. rapa (cv. Tobin), B. juncea (cv. Oriental), B. juncea (cv. Lethridge 22A), Medicago saliva, and Oryzae saliva were tested. Initially, 250 mg of seeds from each of the tested species were separately exposed to 800 ml of deionized water in 1 liter plastic beakers (approximately pH 5.5) at 22.degree.-25.degree. C. The seed-containing aqueous compositions were aerated using sparging stones connected to laboratory air pumps. Seedlings were allowed to develop in the beakers for seven days (except the rice seedlings, which were grown for 12 days in tap water), with the water in each beaker being replaced at the end of day 3. On day 7, seedlings were separately transferred to large plastic tubs. Each tub contained 10 liters of a solution of metals as described in Example 1. Seedlings were incubated in the solution of metals with continuous aeration for 48 hours in the dark at a temperature of 22.degree.-25.degree. C. Each

solution of metals was replaced with a fresh solution of metals after 24 hours.

Detailed Description Text (20):

Another embodiment of the invention contemplates methods for the remediation of metal-containing solutions using dead or inviable plant seedlings. Initially, seedlings (*B. juncea* cv. Lethridge 22A) were germinated and grown in tap water in the dark at 22.degree. C. for 7 days with constant aeration. The biomass of seedlings was then divided into two groups. One group of seedlings was killed by drying at 55.degree. C. for 24 hours. Each group of seedlings was then separately transferred to large plastic tubs. Each tub contained 10 liters of a solution of metals, as defined in Example 1. Each group of seedlings was incubated for 24 hours at 22.degree. C. in the dark. The pH of the solution of metals prior to addition of the seedlings ranged from 6.0-6.5 and did not change during the course of the experiment. Following contact with the solution of metals, seedlings were removed, placed in separate paper envelopes, and dried for 24-48 hours at 80.degree. C. Dried seedlings were then transferred to 50 ml glass digestion tubes and 5 ml of concentrated nitric acid was added. Samples were incubated for 6 hours at room temperature. Subsequently, the samples were incubated for 20 minutes at 180.degree. C. After the samples had cooled, 1 ml of concentrated perchloric acid was added and the samples were again incubated for 20 minutes at 180.degree. C. Deionized water was then added to bring the final volume to 25 ml. Samples were analyzed by Inductively coupled Plasma Spectroscopy using a Fisons Accuris E system. The data are presented in Table 4 as mean bioaccumulation coefficients (three trials), with standard deviations noted parenthetically.

Detailed Description Text (41):

Mutagenesis may be accomplished by exposing dry, or pre-soaked, seeds to EMS in solution at room temperature. Approximately 1.0 g and 5.4 g of *B. juncea* 426308 seeds are placed in two 400 ml plastic beakers. Next, 10 ml of a 0.1M phosphate buffer (3:2 ratio of 0.1M Na₂HPO₄ : 0.1M KH₂PO₄, pH 6) is added to the container with 1.0 g of seeds and 54 ml of the same buffer is added to the container with 5.4 g of seeds. Both beakers are incubated in a rotating incubator set to 150 rpm and room temperature for 3 hours. The buffer in each beaker is replaced with fresh phosphate buffer and, in a fume hood, EMS is added to a final concentration of 160 mM for the beaker containing 5.4 g of seeds (Sigma Chemical Co., St. Louis, Mo, catalog number M-0880). One of ordinary skill in the art will recognize that concentrations of EMS effective in mutagenesis will vary, depending, for example, on the duration of seed pre-soaking, the duration of seed exposure to EMS, and temperature. A preferred concentration of EMS is that concentration resulting in approximately 50% seed viability, as determined by routine experimentation.

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Dec 29, 1998

US-PAT-NO: 5853576

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TITLE: Phytorecovery of metals using seedlings

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kapulnik; Yoram	Highland Park	NJ		
Ensley; Burt	Newtown	PA		
Raskin; Ilya	Manalapan	NJ		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Phytotech, Inc.	Monmouth Junction	NJ			02

APPL-NO: 08/ 911655 [PALM]

DATE FILED: August 15, 1997

PARENT-CASE:

This is a Division of U.S. application Ser. No. 08/602,078, filed Feb. 15, 1995, now U.S. Pat. No. 5,723,300.

INT-CL: [06] C02 F 3/32

US-CL-ISSUED: 210/150, 210/170, 210/602, 210/198.1, 210/251, 47/63, 47/59
US-CL-CURRENT: 210/150, 210/170, 210/198.1, 210/251, 210/602, 47/59R, 47/63

FIELD-OF-SEARCH: 47/60, 47/61, 47/63, 47/64, 210/150, 210/151, 210/170, 210/602, 210/747, 210/610, 210/611, 210/620, 210/631, 210/911-913, 210/198.1, 210/251

PRIOR-ART-DISCLOSED:

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 [Search Selected](#) [Search ALL](#)

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ART-UNIT: 174

PRIMARY-EXAMINER: Wyse; Thomas G.

ABSTRACT:

Methods and systems for alleviating the environmental and health hazards associated with environmental contamination by metals are provided. Contained living or non-viable biomasses of metal-accumulating plant seedlings deplete the metal elements and compounds in metal-containing aqueous solutions. Concomitantly, the contained biomasses of plant seedlings accumulate the metal elements and compounds. The energy and nutrient stores of the seeds from which the seedlings develop are exploited to minimize the costs of remediating the metal-containing aqueous solutions.

12 Claims, 15 Drawing figures

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L12: Entry 4 of 6

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TITLE: Phytorecovery of metals using seedlings

DATE-ISSUED: December 29, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Ensley; Burt	Newtown	PA		
Raskin; Ilya	Manalapan	NJ		

US-CL-CURRENT: 210/150; 210/170, 210/198.1, 210/251, 210/602, 47/59R, 47/63

CLAIMS:

We claim:

1. A system to effect a depletion of metal in a metal-containing solution comprising:
 - (a) chamber means containing a biomass of etiolated metal-accumulating plant seedlings, said seedlings not requiring external nutrients or external energy in the form of light or heat beyond the requirements to achieve normal germination temperatures prior to or during contact with a metal-containing solution; and
 - (b) means for contacting said contained biomass of plant seedlings with said metal-containing solution comprising an inlet means for introducing said metal-containing solution into said chamber means and an outlet means for separating a metal-depleted solution from said contained biomass of plant seedlings.
2. The system according to claim 1 wherein said metal-accumulating plant seedlings do not require external energy.
3. The system according to claim 1 wherein said biomass of metal-accumulating plant seedlings is non-viable.
4. The system according to claim 1 wherein said contacting means immerses said metal-accumulating plant seedlings in said metal-containing solution.
5. The system according to claim 1 wherein said biomass is selected from the group consisting of seedlings of *Brassica napus*, *Brassica rapa*, *Brassica juncea*, *Medicago sativa*, and *Oryzae sativa* seeds.
6. The system according to claim 1 further comprising means associated with said contacting means for aerating said contained biomass of plant seedlings.
7. The system according to claim 6 further comprising means associated with

said contacting means for mixing said contained biomass of plant seedlings.

8. The system according to claim 1 wherein said chamber means comprises a plurality of screen elements for physically segregating portions of said contained biomass of plant seedlings.

9. A system according to claim 5, 6, 7, or 8 comprising a plurality of chamber means and a plurality of contacting means.

10. A system according to claim 9 wherein each chamber means contains a biomass of plant seedlings of different capacity for accumulating metal.

11. A system according to claim 10 wherein differences in biomass capacity for accumulating metal result from differences in plant species.

12. A system according to claim 10 wherein differences in biomass capacity for accumulating metal result from differences in maturity of plant seedlings of the same species.

Periodic Table of the Elements

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		1 New I.A		2 Original		3 Alkali Metals		4 Alkaline earth Metals		5 Transition metals		6 Lanthanide series		7 Noble gases		8 Actinide series		9 Other Metals		10 Nonmetals		11 C Solid		12 Br Liquid		13 H Gas		14 Tc Superconductor		15 He Gas		16 Ne Gas		17 Ar Gas		18 He Gas																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
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L12: Entry 1 of 6

File: USPT

DOCUMENT-IDENTIFIER: US 6313374 B1

TITLE: Method of using pelargonium sp. as hyperaccumulators for remediating contaminated soil

US PATENT NO. (1):
6313374

Brief Summary Text (15):

The ability of plants to extract metal ions from soils and accumulate or sequester those metals in their tissues can be tremendously improved by adjusting the pH of the soil and also by the addition of synthetic chelators to the growing media. These two elements increase the release (desorption) of metal ions from soil particles, thereby increasing the availability of those ions to the plant roots, resulting in increased rate of uptake. The limitations of using metal chelators are:

Brief Summary Text (45):

A preferred mode of utilizing scented geraniums (or plant belonging to the Pelargonium sp.) is to plant well-rooted cuttings (1 month old cuttings) in soil (artificial soil mix, soil) which contains one or more of the heavy metal ions. The plants are irrigated biweekly with regular tap water (approximate pH range of water being 5-9). The plants do not need fertilization if a two-week treatment schedule is followed. Otherwise, plants can be fertilized as required. The plants require natural sunlight and normal temperature regimes to perform essential metabolic activities. The plants should be grown (for a minimum) of 2 weeks in the growth medium to allow the plant to uptake metal ions and translocate them to the shoots. The metal-laden shoot and root biomass, after the two-week or other pre-determined treatment period, can be harvested manually or mechanically. The plant material (if left behind after harvest of shoots) can be allowed to grow back in the next cycle, or new cuttings can be planted in the same site. The biomass can be extracted to obtain essential aromatic oils and the oil-extracted biomass can be used for metal extraction and recycling or disposal. These procedures can be varied by one skilled in the art using known techniques in order to use scented geraniums in other soil types. The geraniums may also be grown from seeds or other cuttings or plant parts, as known in the art.

Brief Summary Text (49):

Soil chemistry can be regulated to facilitate plant growth, availability of metals (such as entrapped metals) or metal uptake. The plants' hyperaccumulating ability to uptake and sequester large amounts of metal ions can be enhanced by use of soil treatments or chemicals that make metals in soils more available to the roots of plants, such as metal chelating agents. To enhance the scented geranium plants ability to uptake metal ions from the growth medium, we are utilizing metal-chelating agents such as EDTA, HEDTA, EGTA, DTPA, etc. in the growth medium. Any other suitable chelating agent may also be used in the methods of this invention. These metal chelators solubilize the metal ions and make them easily available to the roots for easy uptake. Among the chelating agents, EGTA and EDTA have been found to be the most effective chelators at enhancing the accumulation of metal by the plants. EDTA is about 10-times less expensive than EGTA, which makes it more attractive for extensive commercial applications in large contaminated sites. We have also modified the pH of the growth medium and determined the effect of pH on the bioavailability of the different metal species. The interaction of pH and chelating agents on bioavailability of metals and the scented geranium plants ability to uptake those metal ions has been investigated.

Detailed Description Text (3):

Three separate experiments were conducted with replicate cutting propagated plants of

scented geraniums to assess their ability to uptake and sequester cadmium in the shoots and roots. In the first experiment, 2 concentration levels of cadmium (Cd1 and Cd2) were selected for treating the scented geraniums [0.6 mg (Cd1) and 2.5 mg (Cd2) of cadmium nitrate dissolved in 250 mL of water per plant; treatments with these concentrations repeated 5 times over a 2 week period; replicate plants were used for each treatment; overall cadmium nitrate fed to each plant being 3 mg (Cd1) and 12.5 mg (Cd2)]. In the second experiment, 2 concentration levels of cadmium (Cd3 and Cd4) were selected for treating the scented geraniums [0.8 mg (Cd3) and 4.2 mg (Cd4) of cadmium nitrate dissolved in 250 mL of water per plant, treatments with these concentrations repeated 4 times in a 2 week period; replicate plants were used for each treatment; overall cadmium nitrate fed to each plant being 3.3 mg (Cd3) and 16.67 mg (Cd4)]. Experiment III was similar to Experiment II except that the plants were watered with alkaline tap water (pH 10.0). In all experiments the plants were harvested after 2 weeks, roots and shoots separated, and dried. Sample preparation consisted of digestion of a known amount of sample in aqua-regia (in both ashed or non-ashed, dried samples), removal of residue by filtration and making up the volume of the filtrate. The samples were analyzed and verified for the concentration of lead by Inductively Coupled Plasma-Emission Spectroscopy (ICP-ES) at Chemisar Laboratories Inc., Guelph, Ontario, Canada and using a Varian Spectra AA-55 Atomic Absorption Spectrophotometer in our laboratory. The data is presented below in Table 1.

Detailed Description Text (6):

Two separate experiments were conducted with replicate cutting propagated plants of scented geraniums to assess their ability to uptake and sequester lead in the shoots. In the first experiment, 2 concentration levels of lead (Pb1 and Pb2) were selected for treating the scented geraniums [6.3 mg (Pb1) and 25 mg (Pb2) of lead nitrate dissolved in 250 mL of water per plant; treatments with these concentrations repeated 5 times in a 2 week period; replicate plants were used for each treatment; overall lead nitrate fed to each plant being 31.5 mg (Pb1) and 125 mg (Pb2)]. In the second experiment, 2 concentration levels of lead (Pb3 and Pb4) were selected for treating the scented geraniums [8.3 mg (Pb3) and 41.7 mg (Pb4) of lead nitrate dissolved in 250 mL of water per plant, treatments with these concentrations repeated 4 times over a 2 week period; replicate plants were used for each treatment; overall lead nitrate fed to each plant being 33.3 mg (Pb3) and 166.7 mg (Pb4)]. Experiment III was similar to Experiment II except that the plants were watered with alkaline tap water (pH 10.0). In all experiments the plants were harvested after 2 weeks, shoots separated, and dried. Sample preparation consisted of digestion of a known amount of sample in aqua-regia (in both ashed or non-ashed, dried samples), removal of residue by filtration and making up the volume of the filtrate. The samples were analyzed and verified for the concentration of lead by Inductively Coupled Plasma-Emission Spectroscopy (ICP-ES) at Chemisar Laboratories Inc., Guelph, Ontario, Canada and using a Varian Spectra AA55 Atomic Absorption Spectrophotometer in our laboratory. The data is presented below in Table 1.

Detailed Description Text (10):

Two separate experiments were conducted with replicate cutting propagated plants of geraniums to assess their ability to uptake and sequester cadmium in the shoots and roots. Five levels of cadmium were selected for treating the scented geranium [6.25 mg (Cd 5), 12.5 mg (Cd 6), 18.75 mg (Cd 7) 25 mg (Cd 8) of cadmium nitrate dissolved in 50 mL water per plant the treatments with these concentrations were repeated daily over a 2 week period; replicates plants were used for each treatment; overall cadmium nitrate fed to each plant being 87.5 mg (Cd 5), 170 mg (Cd 6), 262.5mg (Cd 7) and 300 mg (Cd 8)]. In both experiments the plants were watered with neutral water (pH 7.0). The plants were harvested after 2 weeks; roots and shoots separated, and dried. Sample preparation consisted of digestion of a known amount of sample using a closed teflon vessel method (Topper, 1990). The samples were analyzed using a Varian Spectra AA Atomic Absorption Spectrophotometer in our laboratory. The data is presented below in Table 2.

Detailed Description Text (13):

Two separate experiments were conducted with replicate cutting propagated plants of geraniums to assess their ability to uptake and sequester lead in the shoots and roots. Five levels of lead were selected for treating the scented geranium [25 mg (Pb 5), 37.5 mg (Pb 6), 50 (Pb 7), 62.5 (Pb 8) of lead nitrate dissolved in 50 mL water per plant; treatments with these concentrations were repeated daily over a 2 week period; replicates plants were used for each treatment; overall lead nitrate fed to each plant being 350 (Pb5), 525 mg (Pb 6), 1162 mg (Pb 7) and 875 mg (Pb 8)]. In both experiments the plants were watered with neutral water (pH 7.0). The plants were harvested after 2 weeks; roots and shoots separated, and dried. Sample preparation consisted of digestion

of a known amount of sample following a closed teflon vessel method (Topper, 1990). The samples were analyzed using a Varian Spectra AA Atomic Absorption Spectrophotometer in our laboratory. The data is presented below in Table 2.

Detailed Description Text (16) :

Two separate experiments were conducted with replicate cutting propagated plants of geraniums to assess their ability to uptake and sequester nickel in the shoots and roots. Five levels of nickel were selected for treating the scented geranium [6.25 mg (Ni 5), 12.5 mg (Ni 6), 18.75 mg (Ni 7) 25 (Ni 8) of nickel nitrate dissolved in 50 mL water per plant; treatments with these concentrations were repeated daily over a 2 week period; replicate plants were used for each treatment; overall nickel nitrate fed to each plant being 87.5 mg (Ni 5), 170 mg (Ni 6), 262.5 mg (Ni 7) and 350 mg (Ni 8)]. In both experiments the plants were watered with neutral water (pH 7.0). The plants were harvested after 2 weeks; roots and shoots separated, and dried. Sample preparation consisted of digestion of a known amount of sample following a closed teflon vessel method (Topper, 1990). The samples were analyzed using a Varian Spectra AA Atomic Absorption Spectrophotometer in our laboratory. The data is presented in Table 2.

Detailed Description Text (20) :

Two experiments were conducted with replicate cutting propagated plants of geraniums to assess their ability to uptake and sequester metals in the shoots and roots, when treated with a mixture of cadmium, lead, nickel. In the first experiment, a solution containing 0.8 mg of CdNO₃.₃ +8.3 mg of PbNO₃.₃ +5 mg of NiNO₃.₃ was used for treating the scented geraniums plants; the metals were provided as cadmium, lead and nickel nitrates dissolved in 250 mL water per plant, applied daily over a 10 days period. In the second experiment, a solution containing higher concentrations of metal mixture (provided as nitrates) was used for treatment, viz., 3.12 mg of CdNO₃.₃ +31.25 mg of PbNO₃.₃ +12.5 mg of NiNO₃.₃ treatments with these concentrations were repeated daily over a 10 day period; replicates plants were used for each treatment. The overall cadmium, lead and nickel nitrate fed to each plant being for the first experiment: 8 mg Cd, 83 mg Pb, and 50 mg Ni. For the second experiment the total amount fed was 31.2 mg Cd, 312.5 mg Pb and 125 mg Ni. In both experiments the plants were watered with neutral water (pH 7.0). The plants were harvested after 10 days; roots and shoots separated, and dried. Sample preparation consisted of digestion of a known amount of sample following a closed teflon vessel method (Topper, 1990). The samples were analyzed using a Varian Spectra AA Atomic Absorption Spectrophotometer in our laboratory. The data is presented in Table 3.

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L12: Entry 1 of 6

File: USPT

Nov 6, 2001

US-PAT-NO: 6313374

DOCUMENT-IDENTIFIER: US 6313374 B1

TITLE: Method of using pelargonium sp. as hyperaccumulators for remediating contaminated soil

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
KrishnaRaj; Sankaran	Guelph			CA
Saxena; Praveen K.	Guelph			CA
Perras; Michel R.	Kitchener			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
University of Guelph	Guelph			CA	03

APPL-NO: 09/ 185797 [PALM]

DATE FILED: November 4, 1998

PARENT-CASE:

This application is a regular application under 35, USC .sctn.111(a) and claims priority from U.S. application Ser. No. 60/064,238, filed Nov. 4, 1997 which is incorporated by reference in its entirety.

INT-CL: [07] A01 G 1/00, A01 H 1/00, A01 H 3/00, C12 N 15/82

US-CL-ISSUED: 800/278; 800/294, 47/58.1

US-CL-CURRENT: 800/278; 47/58.1R, 800/294

FIELD-OF-SEARCH: 800/278, 800/294, 47/58.1, 75/711

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

 [Search Selected](#) [Search ALL](#)

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4872985</u>	October 1989	Dinges	210/602
<input type="checkbox"/>	<u>5120441</u>	June 1992	Jackson	210/602
<input type="checkbox"/>	<u>5320663</u>	June 1994	Cunningham	75/432
<input type="checkbox"/>	<u>5364451</u>	November 1994	Raskin	75/710
<input type="checkbox"/>	<u>5393426</u>	February 1995	Raskin	210/602
<input type="checkbox"/>	<u>5668294</u>	September 1997	Meagher	800/205
<input type="checkbox"/>	<u>5917117</u>	June 1999	Ensley et al.	75/711

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
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WO 97/17429	May 1997	WO	
WO 97/45000	December 1997	WO	

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ART-UNIT: 161

PRIMARY-EXAMINER: Campell; Bruce R.

ASSISTANT-EXAMINER: Grunberg; Anne Marie

ABSTRACT:

A process for effectively remediating soils contaminated with individual or mixture of metal ions is described. The process involves utilizing plants of the genus *Pelargonium*, particularly *Pelargonium* sp., to hyperaccumulate metal ions in their roots and shoots. These plants when grown on soils, which contain one or more of the metal ions, individually or in a complex mixture, will uptake the metal ions through their roots and translocate them to the shoots. This process thereby removes the metal ions from the soil. The harvested shoot and root biomass can be used for extraction of essential aromatic oils, and the residual oil-extracted biomass will be available for extraction and recycling of the metals. The process also describes the use of the above said plant(s) for remediating land-farming sites of petroleum industries, which are generally contaminated with a mixture of metal ions and organic contaminants. The plant surpasses all the requirements of an ideal hyperaccumulator such as, robust growth habit, large shoot biomass (primarily leaves), effective root system, ability to survive and uptake of a wide array of metal ions, ability to retain senescing plant parts, in addition to potential economic returns in the form of essential aromatic oils from harvestable biomass.

18 Claims, 0 Drawing figures

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L12: Entry 1 of 6

File: USPT

US-PAT-NO: 6313374
DOCUMENT-IDENTIFIER: US 6313374 B1

TITLE: Method of using pelargonium sp. as hyperaccumulators for remediating contaminated soil

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
KrishnaRaj; Sankaran	Guelph			CA
Saxena; Praveen K.	Guelph			CA
Perras; Michel R.	Kitchener			CA

US-CL-CURRENT: 800/278; 47/58.1R, 800/294

CLAIMS:

We claim:

1. A method for removing one or more species of metal from a growth medium, comprising growing a Pelargonium sp. scented geranium plant in the growth medium for a time period sufficient for the plant root to uptake and hyperaccumulate metal in the root or shoot biomass, wherein the metal is selected from one or more members of the group consisting of lead, cadmium, copper, nickel and zinc.
2. A method according to claim 1, wherein the Pelargonium sp. plant is selected from the group consisting of Pelargonium sp. 'Frensham', Pelargonium sp 'Citrosa' and Pelargonium sp 'Beauty Oak'.
3. A method according to claim 2, wherein the Pelargonium sp. plant is selected from the group consisting of Pelargonium sp. 'Frensham', and Pelargonium sp 'Beauty Oak'.
4. A method according to claim 3, wherein the Pelargonium sp. plant is Pelargonium sp. 'Frensham'.
5. A method according to claim 1, wherein the growth medium comprises solid medium, semi-solid medium, liquid medium or a combination thereof.
6. A method according to claim 5, wherein the growth medium comprises soil, sand, sludge, compost, or artificial soil mix.
7. A method according to claim 5, wherein the growth medium comprises organic contaminants selected from the group consisting of petroleum industry by-products and petroleum industry wastes.
8. A method according to claim 1, wherein the metal comprises cadmium accumulated at a concentration of about 450 mg Cd/kg to 27,500 mg Cd/kg dry weight of the plant.

9. A method according to claim 1, wherein the metal comprises lead accumulated at a concentration of about 1,300 mg Pb/kg to 70,000 mg Pb/kg dry weight of the plant.
10. A method according to claim 1, wherein the metal comprises nickel accumulated at a concentration of about 400 mg Ni/kg to 21,500 mg Ni/kg dry weight of the plant.
11. The method of claim 1, further comprising the step of harvesting one or more parts of the plant, the part being selected from the group consisting of a portion of the root biomass, a portion of the shoot biomass, the entire root biomass, the entire shoot biomass and the entire root and shoot biomass.
12. A method according to claim 11, wherein the portion of the shoot biomass comprises a leaf or a stem.
13. A method according to claim 11, wherein a sufficient portion of the shoot biomass is not harvested to permit continued plant growth.
14. A method according to claim 1, further comprising the steps of harvesting one or more parts of the plant, the part being selected from the group consisting of a portion of the root biomass, a portion of the shoot biomass the entire root biomass, the entire shoot biomass and the entire root and shoot biomass, and extracting essential aromatic oil from the root or shoot biomass.
15. A method according to claim 14, wherein essential aromatic oil is obtained by distillation.
16. A method according to claim 15, wherein the essential aromatic oil is selected from the group consisting of citronellol, geraniol, iso-methane and geranyl formate.
17. The method of claim 1, wherein one or more parts of the plant selected from the root and shoot biomass is harvested and metal in the root or shoot biomass is concentrated.
18. A method according to claim 17, wherein concentration of metal is carried out by a method selected from the group consisting of air drying, dehydrating, ashing, incineration, smelting, aerobic digestion and anaerobic digestion of the residual oil-extracted shoot biomass.

Periodic Table of the Elements

	1	New I _A Original	18 VII _A
1 H Hydrogen 1.00794 1.00794	2	I _A	
2 Li Lithium 6.941 6.941	3	Alkali Metals Li _A	■ Alkali Metals
3 Be Beryllium 9.01262 9.01262	4	Alkaline earth Metals Be _A	■ Alkaline earth Metals
4	5	Lanthanide series	■ Transition metals
5 Na Sodium 22.98970 22.98970	6	6	■ Nonmetals
6 Mg Magnesium 24.3150 24.3150	7	7	■ Noble gases
7 Ca Calcium 40.078 40.078	8	8	
8 Sr Strontium 87.62 87.62	9	9	
9 K Potassium 39.098 39.098	10	10	
10 Rb Rubidium 84.998 84.998	11	11	
11 Cs Cesium 132.911 132.911	12	12	
12 Ba Barium 137.91 137.91	13	13	
13 La Lanthanum 157.9 157.9	14	14	
14 Ce Cerium 140.91 140.91	15	15	
15 Pr Praseodymium 144.91 144.91	16	16	
16 Nd Neodymium 144.91 144.91	17	17	
17 Sm Samarium 150.91 150.91	18	18	
18 Eu Europium 151.91 151.91	19	19	
19 Gd Gadolinium 157.91 157.91	20	20	
21 Tb Thulium 158.91 158.91	21	21	
22 Dy Dysprosium 162.91 162.91	22	22	
23 Ho Hholmium 164.91 164.91	23	23	
24 Er Erbium 167.91 167.91	24	24	
25 Tm Thulium 168.91 168.91	25	25	
26 Yb Ytterbium 173.91 173.91	26	26	
27 Lu Lutetium 174.91 174.91	27	27	
C Solid			
Br Liquid	13	14	15
H Gas	I _A	V _A	V _{IIA}
Tc Synthetic	16	17	18
B	19	20	21
C	21	22	23
N	23	24	25
O	24	25	26
F	25	26	27
Ne	26	27	28
Ar	27	28	29
Kr	28	29	30
Xe	29	30	31
Rn	30	31	32
Ra	31	32	33
Fr	32	33	34
Ce	33	34	35
Pr	34	35	36
Nd	35	36	37
Sm	36	37	38
Eu	37	38	39
Gd	38	39	40
Tb	39	40	41
Dy	40	41	42
Ho	41	42	43
Er	42	43	44
Tm	43	44	45
Yb	44	45	46
Lu	45	46	47
W	46	47	48
Os	47	48	49
Ru	48	49	50
Pt	49	50	51
Ir	50	51	52
Rh	51	52	53
Pd	52	53	54
Ag	53	54	55
Cu	54	55	56
Sn	55	56	57
Bi	56	57	58
Pb	57	58	59
Bi	58	59	60
Po	59	60	61
At	60	61	62
Rn	61	62	63
Ra	62	63	64
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Cs	64	65	66
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L12: Entry 2 of 6

File: USPT

Aug 31, 1999

US-PAT-NO: 5944872

DOCUMENT-IDENTIFIER: US 5944872 A

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 ODP.

TITLE: Method for phytomining of nickel, cobalt and other metals from soil

DATE-ISSUED: August 31, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chaney; Rufus L.	Beltsville	MD		
Angle; Jay Scott	Ellicot City	MD		
Baker; Alan J.M.	Sheffield			GB
Li; Yin-Ming	Potomac	MD		

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NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE	CODE
University of Maryland at College Park	College Park	MD			02	
The United States of America as represented by the Secretary of	Washington	DC			06	
The University of Sheffield	Sheffield			GB	03	

APPL-NO: 08/ 879813 [PALM]

DATE FILED: June 20, 1997

PARENT-CASE:

This is a continuation application of application Ser. No. 08/470,440 filed Jun. 6, 1995 now U.S. Pat. No. 5,711,784.

INT-CL: [06] C22 B 3/18

US-CL-ISSUED: 75/712; 47/58, 210/602
 US-CL-CURRENT: 75/712; 210/602, 47/58.1R

FIELD-OF-SEARCH: 75/712, 47/58, 210/602

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> 5320663	June 1994	Cunningham	75/432
<input type="checkbox"/> 5393426	February 1995	Raskin et al.	
<input type="checkbox"/> 5711784	January 1998	Chaney et al.	75/712

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ART-UNIT: 172

PRIMARY-EXAMINER: Andrews; Melvin

ABSTRACT:

Nickel/cobalt, as well as platinum and palladium metal family members are recovered from soil by growing Brassicaceae plants, specifically *Alyssum* in soil containing nickel/cobalt as well as other metals. The soil is conditioned by maintaining a low pH, low calcium concentration, and the addition of ammonium fertilizer and chelating agents thereto. Nickel accumulation on the order of 2.5 percent or better in above-ground tissues is achieved, which permits recovery of the metal by harvesting the above-ground plant materials, drying, and then combusting the same, to oxidize or vaporize organic materials and recover the metals sequestered therein at 10-20 fold higher concentrations than in the soil, in a form which can be used in conventional Ni refinery or smelting operations.

12 Claims, 0 Drawing figures

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L12: Entry 2 of 6

File: USPT

DOCUMENT-IDENTIFIER: US 5944872 A

TITLE: Method for phytomining of nickel, cobalt and other metals from soil

US PATENT NO. (1):
5944872Abstract Text (1):

Nickel/cobalt, as well as platinum and palladium metal family members are recovered from soil by growing Brassicaceae plants, specifically Alyssum in soil containing nickel/cobalt as well as other metals. The soil is conditioned by maintaining a low pH, low calcium concentration, and the addition of ammonium fertilizer and chelating agents thereto. Nickel accumulation on the order of 2.5 percent or better in above-ground tissues is achieved, which permits recovery of the metal by harvesting the above-ground plant materials, drying, and then combusting the same, to oxidize or vaporize organic materials and recover the metals sequestered therein at 10-20 fold higher concentrations than in the soil, in a form which can be used in conventional Ni refinery or smelting operations.

Brief Summary Text (12):

The identified metal species are accumulated by growing the Alyssum in nickel-rich soil, under specific soil conditions. The conditions include: 1) lowering the soil pH, which increases the phytoavailability of nickel; 2) maintaining low Ca or lowering Ca in the soil by leaching calcium from the soil by appropriate treatments and by use of low Ca, Mg-rich soil amendments; 3) using ammonium containing or ammonium-generating nitrogen fertilizers to improve plant growth and to increase Ni hyperaccumulation due to rhizosphere acidification; and 4) applying chelating agents to the soil to improve nickel uptake by the roots of the hyperaccumulating Alyssum species. Examples of suitable chelating agents include nitrilotriacetic acid (NTA). Other chelating agents commonly used in connection with increasing soil metal mobility for plant uptake include ethylenediaminetetraacetic acid, and ethylene glycol-bis-(.beta.-aminoethyl ether)-N, N-tetraacetic acid. Maintenance of these four soil-conditioning factors will improve nickel hyperaccumulation in Alyssum, in excess of a 2.5 percent concentration in above-ground portions of the plant, particularly leaves and stems, which make for easy cultivation and metal recovery. This is preferable to concentration in the roots, discussed in Raskin et al, which may be an aid in soil remediation if non-leachable therefrom, but does not offer convenience for phytomining.

Detailed Description Text (5):

These include soil pH, low calcium concentrations, use of ammonium containing or generating fertilizer rather than other N-fertilizers and application of chelating agents. Each of these is considered in turn below.

Detailed Description Text (7):

The maintenance of preferred pH ranges in soil is well known in agriculture for a variety of reasons. Typically, pH of soil is altered or modified so as to maintain it within a near neutral range of about 6.0-7.5. Thus, soil near a limestone foundation or other building may be treated with acidifying soil amendments so as to reduce an alkaline pH. Soil with a naturally low pH may instead be treated with limestone or similar amendment, so as to increase the soil pH. A reduced pH increases the phytoavailability of nickel and cobalt. A reduced pH increases solubility, and optimizes the release of these metals for absorption by the roots, and translocation to the above-ground tissues of the plant. Soil pH can be maintained in any of a variety of established methods, and the methods themselves do not constitute an aspect of this invention. Preferably, soil pH is managed at a low value by addition of sulfur and use

of ammonium--N fertilizers. The Alyssum species, and indeed, any plant species, grows best at its evolved optimum pH conditions. Thus, pH cannot be reduced so low as to substantially retard or inhibit plant growth. An optimum pH range for phytomining using Alyssum is a pH of 4.5 to 6.2, preferably 5.2-6.2. After extraction of economically phytominable Ni and Co from the soil, limestone application can raise soil to pH levels required by more traditional farm crops.

CLAIMS:

recovering the nickel from said harvested biomass materials, wherein said soil is conditioned by maintaining the pH of the soil at within a range of 4.5 to 6.2.

5. The method of claim 4, wherein said soil is conditioned by maintaining the pH of the soil at within a range of 4.5 to 6.2.

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L12: Entry 2 of 6

File: USPT

US-PAT-NO: 5944872

DOCUMENT-IDENTIFIER: US 5944872 A

TITLE: Method for phytomining of nickel, cobalt and other metals from soil

DATE-ISSUED: August 31, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chaney; Rufus L.	Beltsville	MD		
Angle; Jay Scott	Ellicot City	MD		
Baker; Alan J.M.	Sheffield			GB
Li; Yin-Ming	Potomac	MD		

US-CL-CURRENT: 75/712; 210/602, 47/58.1R

CLAIMS:

What is claimed is:

1. A method of recovering nickel from soil, comprising:

cultivating Alyssum plants in soil containing nickel, under conditions sufficient to permit said Alyssum to accumulate nickel from the soil in above-ground tissues of said Alyssum;

harvesting said Alyssum as biomass materials after accumulation of nickel from the soil, and;

recovering the nickel from said harvested biomass materials, wherein said soil is conditioned by maintaining the pH of the soil at within a range of 4.5 to 6.2.

2. The method of claim 1, wherein said soil, has an exchangeable calcium concentration and an exchangeable Mg concentration, and wherein the exchangeable calcium concentration is managed such that it has a value lower than 20% of the exchangeable Mg concentration, adding ammonium-containing fertilizer to said soil and adding chelating agents to said soil.

3. The method of claim 1, wherein said metal is recovered by drying and combusting, said harvested biomass materials, to oxidize and vaporize organic materials present.

4. A method of recovering nickel from soil, comprising:

cultivating Alyssum plants in soil containing nickel, under conditions sufficient to permit said Alyssum to accumulate nickel from the soil in above-ground tissues of said Alyssum such that at least 2.5% of the air-dried above-ground tissue of said Alyssum is nickel;

harvesting said Alyssum as biomass materials after accumulation of nickel from

the soil, and;

recovering the nickel from said harvested biomass materials.

5. The method of claim 4, wherein said soil is conditioned by maintaining the pH of the soil at within a range of 4.5 to 6.2.

6. The method of claim 5, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration, and wherein the exchangeable calcium concentration is managed such that it has a value lower than 20% of the exchangeable Mg concentration.

7. The method of claim 6, wherein ammonium-containing fertilizer is added to said soil.

8. The method of claim 7, wherein chelating agents are added to said soil.

9. A method of recovering nickel from soil, comprising:

cultivating Alyssum plants in soil containing nickel, under conditions sufficient to permit said Alyssum to accumulate nickel from the soil in above-ground tissues of said Alyssum, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration and wherein the exchangeable calcium concentration is managed such that it has a value lower than 20% of the exchangeable Mg concentration;

harvesting said Alyssum as biomass materials after accumulation of nickel from the soil, and;

recovering the nickel from said harvested biomass materials.

10. The method of claim 1, wherein said Alyssum plants are selected from the group consisting of *A. murale*, *A. pintodasilvae*, *A. malacitanum*, *A. lesbiacum*, *A. fallacinum*, *A. argentum*, *A. bertolonii*, *A. tenium*, *A. heldriechii*, and mixtures thereof.

11. The method of claim 4, wherein said Alyssum plants are selected from the group consisting of *A. murale*, *A. pintodasilvae*, *A. malacitanum*, *A. lesbiacum*, *A. fallacinum*, *A. argentum*, *A. bertolonii*, *A. tenium*, *A. heldriechii*, and mixtures thereof.

12. The method of claim 9, wherein said Alyssum plants are selected from the group consisting of *A. morale*, *A. pintodasilvae*, *A. malacitanum*, *A. lesbiacum*, *A. fallacinum*, *A. argentum*, *A. bertolonii*, *A. tenium*, *A. heldriechii*, and mixtures thereof.

Periodic Table of the Elements

1 IA Original		2 IIA		3 III A		4 IVA		5 VA		6 VI A		7 VIIA		18 VIIIA			
1 H Hydrogen 1.0084		2 Li Lithium 6.9387		3 Na Sodium 22.98976		4 Mg Magnesium 24.3580		5 Al Aluminum 26.98153		6 Si Silicon 28.0855		7 P Phosphorus 30.97376		8 S Sulfur 32.06		9 Cl Chlorine 35.4527	
10 Be Beryllium 9.01287		11 B Boron 10.8114		12 C Carbon 12.0107		13 N Nitrogen 14.01		14 O Oxygen 15.9994		15 F Fluorine 18.9984		16 Ne Neon 20.1797		17 He Helium 2.0197			
18 VIIIA		19 K Potassium 39.0984		20 Ca Calcium 40.078		21 Sc Scandium 44.9559		22 Ti Titanium 47.867		23 V Vanadium 50.9420		24 Cr Chromium 51.9961		25 Mn Manganese 54.9386		26 Fe Iron 55.8470	
27 Rb Rubidium 85.462		28 Sr Strontium 87.621		29 Y Yttrium 88.9058		30 Zr Zirconium 91.224		31 Nb Niobium 92.9063		32 Ta Tantalum 97.9078		33 W Tungsten 183.84		34 Ru Ruthenium 190.23		35 Rh Rhodium 196.967	
36 Cs Cesium 132.911		37 Ba Barium 137.327		38 La Lanthanum 138.9055		39 Hf Hafnium 178.49		40 Ta Tantalum 180.9552		41 Re Rhenium 186.207		42 Os Osmium 190.23		43 Ir Iridium 192.217		44 Pt Platinum 195.084	
45 Fr Francium 223		46 Ra Radium 226		47 Rf Rutherfordium 227		48 Ds Darmstadtium 229		49 Sg Sgmevium 229		50 Bh Bhmevium 229		51 Hs Hsmevium 229		52 Mt Mevium 229		53 Uuu Ununtrium 229	
54 Uub Ununpentium (229)		55 Uup Ununpentium (229)		56 Uus Ununseptium (229)		57 Uuo Ununoctium (229)		58 Uup Ununpentium (229)		59 Uus Ununpentium (229)		60 Uuo Ununoctium (229)		61 Uup Ununpentium (229)		62 Uus Ununpentium (229)	
63 Uuo Ununoctium (229)		64 Gd Gadolinium 157.25		65 Dy Dysprosium 162.50		66 Ho Holmium 164.932		67 Er Erbium 167.26		68 Tm Thulium 169.932		69 Yb Ytterbium 173.00		70 Lu Lutetium 174.967		71 Yb Ytterbium (229)	
72 Pa Protactinium 231.03592		73 U Uranium 231.03592		74 Nb Niobium 231.03592		75 Sn Tin 231.03592		76 Eu Europium 231.03592		77 Gd Gadolinium 231.03592		78 Dy Dysprosium 231.03592		79 Ho Holmium 231.03592		80 Er Erbium 231.03592	
81 Pa Protactinium 232.03592		82 U Uranium 232.03592		83 Nb Niobium 232.03592		84 Sn Tin 232.03592		85 Eu Europium 232.03592		86 Gd Gadolinium 232.03592		87 Dy Dysprosium 232.03592		88 Ho Holmium 232.03592		89 Er Erbium 232.03592	
90 Pa Protactinium 232.03592		91 U Uranium 232.03592		92 Nb Niobium 232.03592		93 Sn Tin 232.03592		94 Eu Europium 232.03592		95 Gd Gadolinium 232.03592		96 Dy Dysprosium 232.03592		97 Ho Holmium 232.03592		98 Er Erbium 232.03592	
99 Pa Protactinium 232.03592		100 U Uranium 232.03592		101 Nb Niobium 232.03592		102 Sn Tin 232.03592		103 Eu Europium 232.03592		104 Gd Gadolinium 232.03592		105 Dy Dysprosium 232.03592		106 Ho Holmium 232.03592		107 Er Erbium 232.03592	

Atomic masses in parentheses are those of the most stable or common isotope.

Note: The subgroup numbers 1-18 were adopted in 1984 by the International Union of Pure and Applied Chemistry. The names of elements 110-118 are the Latin equivalents of those numbers.

Weight-Volume Properties of Solids, Liquids, and Gases																
1 IA	2 IIA	3 III A	4 IVA	5 VA	6 VI A	7 VIIA	8 VIIIA	9 IA	10 IIA	11 III A	12 IVA	13 VA	14 VI A	15 VIIA	16 VIIIA	
C Solid	Br Liquid	H Gas	Tc Superconductor	B Diamond Graphite	C Graphite Diamond	N Diamond Graphite	P Diamond Graphite	S Diamond Graphite	Cl Diamond Graphite	Ar Diamond Graphite	Ge Diamond Graphite	As Diamond Graphite	Se Diamond Graphite	Kr Diamond Graphite	Xe Diamond Graphite	Rn Diamond Graphite
17 He Gas	18 Ne Gas	19 Ar Gas	20 Kr Gas	21 Xe Gas	22 Rn Gas	23 Fr Gas	24 Ra Gas	25 Ac Gas	26 Rf Gas	27 Ds Gas	28 Sg Gas	29 Bh Gas	30 Hs Gas	31 Mt Gas	32 Uuu Gas	
33 Sc Solid	34 Ge Solid	35 As Solid	36 Pt Solid	37 Bi Solid	38 Po Solid	39 At Solid	40 Ru Solid	41 Rh Solid	42 Pd Solid	43 Rh Solid	44 Ru Solid	45 Pt Solid	46 Ir Solid	47 Pt Solid	48 Ru Solid	

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not a prior art

L12: Entry 3 of 6

File: USPT

DOCUMENT-IDENTIFIER: US 5917117 A
TITLE: Inducing hyperaccumulation of metals in plant shoots

US PATENT NO. (1):
5917117

Abstract Text (1):

The present invention provides methods by which hyperaccumulation of metals in plant shoots is induced by exposure to inducing agents. Hyperaccumulation occurs as part of a two-step process in which metals are first accumulated into plant roots; subsequent transport to plant shoots is induced by exposure to the agent. In preferred embodiments, manipulations that increase availability of metals to the plant are employed prior to application of the inducing agent. Effective inducing agents include conditions of low pH, chelators, herbicides, and high levels of heavy metals. Other phytotoxic agents are also useful. Application of multiple inducing agents results in synergistic effects.

Drawing Description Text (3):

FIG. 2 is a bar graph showing the effects of acidification on lead accumulation in roots and shoots of a *Brassica juncea* cultivar after acidification to pH 3.5.

Detailed Description Text (9):

The present invention also demonstrates that combinations of inducing agents, applied simultaneously or with intervening time periods, often have synergistic effects on metal accumulation. In preferred embodiments of the invention, plants are exposed to a first manipulation that increases metal availability (e.g., by employing a first inducing agent that itself increases metal availability and/or by taking additional steps to enhance availability, as is discussed below), and then to a second manipulation comprising application of an inducing agent that stimulates metal transport to the shoots. For example, we have found the application of low pH and/or a chelating agent as a first inducing agent, followed by a delay period and application of herbicide as a second inducing agent, results in very high levels of metal hyperaccumulation. It is particularly preferred that plants be cultivated to high biomass prior to exposure to the first or second manipulations, in order that a large volume of plant tissue is available for metal accumulation. It may also be desirable, however, for accumulation to be induced prior to termination of plant growth.

Detailed Description Text (38):

The term "increase the availability of metal", as used herein, refers to rendering metals in an environment more amenable to plant root uptake, and/or to subsequent shoot transport, than they would be in the absence of the manipulation. Manipulations that can increase the availability of metal to plants include, for example, (i) addition of chelators to the soil; (ii) tilling of soil to bring metal containing soil into contact with the plant root zone; (iii) decreasing pH of the metal-containing environment, for example by adding an effective amount of an organic or inorganic acid (such as, for example, nitric acid, acetic acid, and citric acid), or by adding to the environment a compound, such as ammonium sulfate, that will be metabolized by the plant roots (and/or by associated bacteria or other component(s) of the rhizosphere) in a manner that produces protons and thereby reduces the soil pH (see, for example, U.S. Ser. No. 08/252,234, incorporated herein by reference; see also Example 10).

Detailed Description Text (45):

As discussed above, chelators such as EDTA improve metal solubility in the soil, and thereby increase availability of the soil metals to the plant. This increase in metal solubility presumably increases the amount of metal accumulated in the plant. However,

the evidence presented in Examples 1 and 2 shows clearly that EDTA has an effect on metal accumulation into shoots that is beyond any effect it has on metal availability because the observed hyperaccumulation of lead into plant shoots does not increase linearly with EDTA concentration, as would be expected for a solubility effect. Rather, lead uptake increases dramatically above a threshold level (greater than about 0.3 mmol/kg at pH 5.1 and greater than about 1.0 mmol/kg at pH 7.5 in Example 2). Thus, we have demonstrated that EDTA induces hyperaccumulation of lead into plant shoots by stimulating transport of root-accumulated material.

Detailed Description Text (49):

Example 3 reports our finding that exposure of *B. juncea* plants to pH 3.5 in solution culture induces hyperaccumulation of lead in plant shoots. We also present data in Example 4 demonstrating that the sequential administration of an acid and EDTA induces higher levels of lead accumulation in *B. juncea* shoots than are induced by administration of either the acid or EDTA alone. Furthermore, Example 5 demonstrates that a combination of acid and EDTA induces metal transport into shoots effectively in a field environment. This finding is particularly significant because large-scale acidification of soil to pH 3.5 may well be impractical in soil sites. The data presented in Example 5 demonstrate that such large-scale acidification is not required. Some level of acidification (we note that the quantities of acid used in Example 5 only slightly reduce the soil pH) is still valuable due to its synergistic effects when combined with another inducing agent such as a chelator.

Detailed Description Text (50):

We note that standard techniques of plant cultivation in soils recommend that pH be maintained between about pH 5.5 and pH 7.0 for optimum growth of most crops. In fact, a large literature has developed that describes how best to treat different types of soil to ensure that a desirable pH is maintained (see, for example, Commercial Vegetable Production Recommendations, Reiners and Garrison, eds., Rutgers, State University of New Jersey, 1994, pp. 18-27; "Agronomy of Canola in the United States", pp. 25-35 in Canola and Rapeseed, Production, Chemistry, Nutrition, and Processing Technology, ed. F. Shahidi, Van Nostrand Reinhold, New York, 1990, each of which is incorporated herein by reference).

Detailed Description Text (51):

Thus, according to the present invention, a soil pH greater than about 5.5 is desirable in the initial cultivation stage during which most of the biomass is accumulated. This initial cultivation stage is followed by a reduction in pH to induce metal accumulation. As described in the Examples, soil pH is preferably reduced to about pH 3.5, though less dramatic pH reductions are also desirable, especially when an additional inducing agent is employed. In fact, any acidification (either localized or general) of the soil-root system is expected to be beneficial to the induction mechanism when used in combination with other inducing agents, regardless of its ability to stimulate induction in the absence of other inducing agents.

Detailed Description Text (52):

The principles exemplified by the data in Examples 3 and 5 are, of course, not limited to *B. juncea* nor to the precise cultivation and/or induction conditions described. For example, different pH ranges may be optimal for induction in different plants. One of ordinary skill in the art can readily follow the teachings of the present specification to screen different plants and conditions and identify those combinations that result in induction of hyperaccumulation in plant shoots.

Detailed Description Text (53):

Also, as reported in the Examples, solution pH was reduced by application of 1.0 N HNO₃. Alternate acidifying agents (such as, for example, acetic acid, ammonium acetate, ammonium sulfate, ferrous sulfate, ferrous sulfide, elemental sulfur, sulfuric acid, citric acid, ascorbic acid) can be used to reduce the soil pH. Also, soil pH can be reduced by addition of a metabolite that is processed by the roots or other element of the rhizosphere in a manner that produces protons (see above). Preferred acidifying agents are those that chemically or biologically degrade within days or weeks without leaving residual salts that may either result in an undesirable buildup of salinity (i.e., ammonium, chloride or sodium) or create a potential environmental hazard from leaching of the associated anions (i.e., nitrate from nitric acid). Particularly preferred acidifying agents include, but are not limited to, acetic acid, citric acid, or ascorbic acid.

Detailed Description Text (60):

The present invention therefore teaches that exposing plants to a physiological stress

or phytotoxic substance (e.g. phytotoxic levels of metals or nutrients, low pH, osmotic stress, herbicide, etc.) or combination of such substances, disrupts the plant's natural safety mechanisms normally involved in preventing uptake and/or transport of toxic substances into plant shoots and stimulates metal translocation from the roots to the shoots. Thus, according to the present invention, any agent with phytotoxic activity can be screened to test its ability to induce metal hyperaccumulation in plant shoots according to the procedures described herein.

Detailed Description Text (80):

A Sassafras Ap silt loam soil was collected from the Rutgers University Horticultural Farm and amended with lead carbonate. The soil was limed to pH 5.1 or 7.5, and was fertilized with urea (150 mg N/kg), potassium chloride (100 mg KCl/kg), and gypsum (70 mg CaSO₄/kg). The soil was allowed to equilibrate for two weeks in the greenhouse at saturation, air dried, and remixed before planting. The soil was placed in 8.75 cm diameter pots (350 g soil/pot) and planted with *Brassica juncea* (426308) seeds. Phosphate fertilizer was added as a spot placement 1 cm below the seeds at planting at the rate of 100 mg P₂O₅/kg. After seedling emergence, the pots were thinned to two plants per pot.

Detailed Description Text (86):

Inducing Hyperaccumulation of Lead by Altering pH

Detailed Description Text (90):

After three weeks, plants were rinsed in deionized water for 20 minutes and then transferred to a container with 750 mL of contaminated solution. Lead nitrate was used to obtain 50 mg Pb/L solution. Solution concentration remained constant for the duration of experiment. Solution pH was adjusted to either pH 5.5 or pH 3.5 by addition of 1.0 N HNO₃. Plants were exposed to the lead contaminated solution, under the low-pH conditions, for 7 days, and then were harvested.

Detailed Description Text (94):

Results are presented in FIG. 2. As can be seen, reducing the pH of the contaminated solution from 5.5 to 3.5 dramatically changed the amount of lead taken up by *B. juncea* shoots. Plants exposed to 50 mg/L lead solution at a pH of 3.5 accumulated 6 mg/g lead, some 100 times the amount taken up at a pH of 5.5. This phenomenon cannot be explained by increased lead solubility, since the soluble lead remained at 50 mg/L during the entire experimental period at either pH level.

Detailed Description Text (96):

Synergistic Induction of Lead Hyperaccumulation by Exposure to a Sequence of Altered pH and EDTA

Detailed Description Text (98):

Experiments were performed as described above in Example 3 except that, after the plants were exposed to the lead-containing solution at the adjusted pH, EDTA was added. Four different reaction conditions were utilized:

Detailed Description Text (99):

a. Control: pH=5.5, no EDTA addition

Detailed Description Text (100):

b. pH=5.5, EDTA added at 0.2 mM

Detailed Description Text (101):

c. pH=3.5, no EDTA addition

Detailed Description Text (102):

d. pH=3.5, EDTA added at 0.2 mM

Detailed Description Text (103):

pH of the solutions was adjusted using a 1.0 N HNO₃ solution. EDTA was added after pH adjustment using 0.5 molar stock solution. At least 4 replicates were used for each treatment.

Detailed Description Text (105):

Results are presented in FIG. 3. As can be seen, the combination of low pH (3.5) and EDTA application has a synergistic effect. The sequence of pH adjustment to 3.5 followed by a dosage of EDTA results in hyperaccumulation levels much higher than the use of a single addition of EDTA or of acid. The lead concentration in dried shoots of

1.7% and the corresponding bioaccumulation coefficient.sup.1 of 340 achieved with the combination of pH 3.5 and addition of EDTA are higher than any values reported in Examples 1-3.

Detailed Description Text (154):

Also as discussed above, metal mobility in soil can be increased by decreasing the soil pH. Conventional methods of plant cultivation generally require soil in the pH range 5.8-6.2 for optimum production and the available literature suggests that soils with lower pH be specifically amended with base (e.g., lime) prior to seeding to increase the pH (see, for example, "Agronomy of Canola in the United States", pp. 25-35 in Canola and Rapeseed, Production, Chemistry, Nutrition, and Processing Technology, ed. F. Shahidi, Van Nostrand Reinhold, New York, 1990, incorporated herein by reference).

Detailed Description Text (155):

In order to increase metal availability in the practice of the present invention, however, pH of the metal-contaminated soil is reduced to about pH 4.5-5.5 by acidifying the soil with an effective amount of organic or inorganic acids (such as nitric acid, hydrochloric acid, sulfuric acid, acetic acid and citric acid). Acids are preferably applied to the soil by conventional irrigation pipes or other ground level irrigation systems. Acids may alternately be applied through other commercially available fertilizer and chemical application equipment, including large volume sprayers. Acids are preferably applied at concentrations from 0.1 mM to 1.0 M at volumes ranging from about 5 to 200 tons per acre or at levels sufficient to drop soil pH in the plant rhizosphere (down to about 40 cm) to between 4.5 and 5.5 pH units.

Detailed Description Text (156):

Acidification of the plant environment may alternately be accomplished by addition to the environment of compounds that depress soil pH because of biological activity of roots and microorganisms. Examples of these compounds include urea or ammonium sulfate. This so-called "biological acidification" occurs because the positively charged ammonium ions that are incorporated into the roots and/or microorganisms are replaced with positively charged protons exuded or otherwise released from the rhizosphere into the soil, thus lowering the soil pH. The ammonium-containing compounds are applied at 0.5 to about 2.0 tons per acre.

Detailed Description Text (157):

Where acidification techniques are employed in combination with chelators, it is generally desirable to reduce the soil pH by at least 2 units over a period of several days, preferably to a pH within the range of about 3-4.5, by adding strong chelators or acids prior to harvest but after the plants have reached the harvestable stage.

Detailed Description Paragraph Table (1):

		Pb uptake by the shoots of B. juncea																
		from soil amended with EDTA. EDTA Pb Uptake into Shoots mmol/kg .mu.g/g																
		Sassafras Ap soil (pH 5.1) 0.3 917 .+- 221 0.5																
3066	.+-.	1362	1.0	6748	.+-.	1842	2.5	8162	.+-.	2501	5.0	11740	.+-.	3802	7.5	15321	.+-.	
1491	Sassafras	Ap	soil	(pH	7.5)	0.0	15	.+-.	1	1.0	243	.+-.	35	2.5	1398	.+-.	560	5.0
5590	.+-.	1916																

CLAIMS:

19. The method of claim 1 wherein the step of manipulating comprises reducing the soil pH to about pH 3.0-5.5.
20. The method of claim 19 wherein the step of manipulating comprises reducing soil pH through application of an effective amount of an organic or inorganic acid selected from the group consisting of nitric acid, hydrochloric acid, sulfuric acid, acetic acid, and citric acid.
21. The method of claim 19 wherein the step of manipulating comprises reducing soil pH through application of a compound that is metabolized by the plant rhizosphere in a manner that produces protons.
34. The method of claim 29 wherein the step of exposing comprises exposing the plant to reduced pH conditions by adding to the soil a metabolite that is processed by elements of the plant rhizosphere in a manner that produces protons.
35. The method of claim 33 wherein the step of exposing comprises exposing the plant to a soil pH below about pH 5.0.

36. The method of claim 35 wherein the step of exposing comprises exposing the plant to a soil pH below about pH 3.5.

55. The method of claim 53 wherein the plant is a *Brassica juncea* cultivar, the metal is lead, and the inducing agent is selected from the group consisting of at least 0.2 mM EDTA, pH less than about 3.5, and an herbicide selected from the group consisting of glyphosate, 2,4-D, and combinations thereof.

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L12: Entry 3 of 6

File: USPT

Jun 29, 1999

US-PAT-NO: 5917117

DOCUMENT-IDENTIFIER: US 5917117 A

TITLE: Inducing hyperaccumulation of metals in plant shoots

DATE-ISSUED: June 29, 1999

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APPL-NO: 08/ 621138 [PALM]

DATE FILED: March 21, 1996

INT-CL: [06] C22 B 3/24

US-CL-ISSUED: 75/711; 75/712, 210/602

US-CL-CURRENT: 75/711; 210/602, 75/712

FIELD-OF-SEARCH: 75/710, 75/711, 75/712, 210/602

PRIOR-ART-DISCLOSED:

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 [Search Selected](#) [Search ALL](#)

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- Salt et al., "Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants", *Bio/Technology*, 13:468, May, 1995.
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ART-UNIT: 172

PRIMARY-EXAMINER: Andrews; Melvyn

ABSTRACT:

The present invention provides methods by which hyperaccumulation of metals in plant shoots is induced by exposure to inducing agents. Hyperaccumulation occurs as part of a two-step process in which metals are first accumulated into plant roots; subsequent transport to plant shoots is induced by exposure to the agent. In preferred embodiments, manipulations that increase availability of metals to the plant are employed prior to application of the inducing agent. Effective inducing agents include conditions of low pH, chelators, herbicides, and high levels of heavy metals. Other phytotoxic agents are also useful. Application of multiple inducing agents results in synergistic effects.

57 Claims, 6 Drawing figures

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L12: Entry 3 of 6

File: USPT

US-PAT-NO: 5917117

DOCUMENT-IDENTIFIER: US 5917117 A

TITLE: Inducing hyperaccumulation of metals in plant shoots

DATE-ISSUED: June 29, 1999

INVENTOR-INFORMATION:

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US-CL-CURRENT: 75/711; 210/602, 75/712

CLAIMS:

What is claimed is:

1. A method of inducing hyperaccumulation of a metal into shoots of a plant comprising;

planting a plant in a soil environment contaminated with one or more metals;

manipulating the soil environment to increase chemical availability of metals in the environment to the plant;

cultivating the plant in the manipulated soil environment under conditions and for a time sufficient for the plant to accumulate metal in its roots; and

exposing the plant to an inducing agent under conditions and for a time sufficient for the inducing agent to induce the plant to hyperaccumulate metal in its shoots.

2. The method of claim 1 further comprising a step of harvesting the plant shoots into which metal has been accumulated.

3. The method of claim 1 or 2 wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates more metal in its shoots than it would accumulate in the absence of the inducing agent.

4. The method of claim 3 wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about twice as much metal in its shoots as it would accumulate in the absence of the inducing agent.

5. The method of claim 3 wherein the step of planting comprises planting a plant in a soil environment contaminated with one or more metals selected from

the group consisting of aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, gold, lead, manganese, mercury, molybdenum, nickel, palladium, selenium, silver, strontium, tin, uranium, vanadium, zinc, zirconium and combinations thereof with one another or with an organic contaminant.

6. The method of claim 5 wherein the step of planting comprises planting a plant in a soil environment contaminated with a metal that is not essential for plant growth.

7. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with a metal selected from the group consisting of cadmium, chromium, copper, lead, nickel, and zinc.

8. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with cadmium and the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 500 .mu.g cadmium/g dry weight plant tissue.

9. The method of claim 8, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 1000 .mu.g cadmium/g dry weight plant tissue.

10. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with copper and the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 1000 .mu.g copper/g dry weight plant tissue.

11. The method of claim 10, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 2500 .mu.g copper/g dry weight plant tissue.

12. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with lead and the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 3000 .mu.g lead/g dry weight plant tissue.

13. The method of claim 12, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 4000 .mu.g lead/g dry weight plant tissue.

14. The method of claim 12, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 6000 .mu.g/g lead/g dry weight plant tissue.

15. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with nickel and the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 200 .mu.g nickel/g dry weight plant tissue.

16. The method of claim 15, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 500 .mu.g nickel/g dry weight plant tissue.

17. The method of claim 5, wherein the step of planting comprises planting a plant in a soil environment contaminated with zinc and the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 1000 .mu.g zinc/g dry weight plant tissue.

18. The method of claim 17, wherein the step of exposing comprises exposing the plant to an inducing agent under conditions and for a time sufficient that the plant accumulates at least about 2000 .mu.g zinc/g dry weight plant tissue.
19. The method of claim 1 wherein the step of manipulating comprises reducing the soil pH to about pH 3.0-5.5.
20. The method of claim 19 wherein the step of manipulating comprises reducing soil pH through application of an effective amount of an organic or inorganic acid selected from the group consisting of nitric acid, hydrochloric acid, sulfuric acid, acetic acid, and citric acid.
21. The method of claim 19 wherein the step of manipulating comprises reducing soil pH through application of a compound that is metabolized by the plant rhizosphere in a manner that produces protons.
22. The method of claim 3 wherein the step of planting comprises planting a plant that is a member of the family Brassicaceae.
23. The method of claim 22 wherein the step of planting comprises planting a plant that is a member of a genus selected from the group consisting of *Brassica*, *Thlaspi*, *Alyssum*, and *Eruca*.
24. The method of claim 23 wherein the step of planting comprises planting a plant that is a member of a species selected from the group consisting of *Brassica juncea*, *Brassica nigra*, *Brassica campestris*, *Brassica carinata*, *Brassica napus*, and *Brassica oleracea*.
25. The method of claim 23 wherein the step of planting comprises planting a plant that is a *Brassica juncea* cultivar.
26. The method of claim 25 wherein the step of planting comprises planting *Brassica juncea* cultivar number 426308.
27. The method of claim 3 wherein the step of exposing comprises exposing the plant to an inducing agent that stimulates metal transport from plant roots to plant shoots.
28. The method of claim 27 wherein the step of exposing comprises exposing the plant to an inducing agent that does not substantially affect metal uptake into plant roots.
29. The method of claim 3 wherein the step of exposing comprises exposing the plant to an inducing agent selected from the group consisting of chelators, soil acidifiers, herbicides, and detergents.
30. The method of claim 29 wherein the step of exposing comprises exposing the plant to a chelator selected from the group consisting of EDTA, EGTA, DTPA, CDTA, HEDTA, NTA, citric acid, salicylic acid, and malic acid.
31. The method of claim 30 wherein the step of exposing comprises exposing the plant to EDTA.
32. The method of claim 31 wherein the step of exposing comprises exposing the plant to a concentration of EDTA greater than about 0.2 mM.
33. The method of claim 29 wherein the step of exposing comprises exposing the plant to a soil acidifier selected from the group consisting of nitric acid, acetic acid, ammonium acetate, ammonium sulfate, ferrous sulfate, ferrous sulfide, elemental sulfur, sulfuric acid, citric acid, and ascorbic acid.

34. The method of claim 29 wherein the step of exposing comprises exposing the plant to reduced pH conditions by adding to the soil a metabolite that is processed by elements of the plant rhizosphere in a manner that produces protons.

35. The method of claim 33 wherein the step of exposing comprises exposing the plant to a soil pH below about pH 5.0.

36. The method of claim 35 wherein the step of exposing comprises exposing the plant to a soil pH below about pH 3.5.

37. The method of claim 32 wherein the step of exposing comprises exposing the plant to an herbicide selected from the group consisting of MCPA, maleic hydrazide, 2,4-D, glyphosate, and combinations thereof.

38. The method of claim 29 wherein the step of exposing comprises exposing the plant to a combination of chelating agent and soil acidifier.

39. The method of claim 38 wherein the chelating agent is selected from the group consisting of EDTA, EGTA, DTPA, CDTA, HEDTA, NTA, citric acid, salicylic acid, and malic acid, and the soil acidifier is selected from the group consisting of nitric acid, acetic acid, ammonium acetate, ammonium sulfate, ferrous sulfate, ferrous sulfide, elemental sulfur, sulfuric acid, citric acid, ascorbic acid, and metabolites that are processed by elements of the plant rhizosphere in a manner that produces protons.

40. The method of claim 29 wherein the step of exposing comprises exposing the plant to a combination of chelating agent and herbicide.

41. The method of claim 40 wherein the chelating agent is selected from the group consisting of EDTA, EGTA, DTPA, CDTA, HEDTA, NTA, citric acid, salicylic acid, and malic acid, and the herbicide is selected from the group consisting of MCPA, maleic hydrazide, 2,4-D, glyphosate, and combinations thereof.

42. The method of claim 41 wherein the step of exposing comprises:
exposing the plant to the chelating agent;
waiting a period of time; and
exposing the plant to the herbicide.

43. The method of claim 29 wherein the step of exposing comprises exposing the plant to an acidifying agent and an herbicide.

44. The method of claim 43 wherein the acidifying agent is selected from the group consisting of nitric acid, acetic acid, ammonium acetate, ammonium sulfate, ferrous sulfate, ferrous sulfide, elemental sulfur, sulfuric acid, citric acid, ascorbic acid, and metabolites that are processed by elements of the plant rhizosphere in a manner that produces protons and the herbicide is selected from the group consisting of glycophosphate, MCPA, maleic hydrazide, 2,4-D, glyphosate, and combinations thereof.

45. The method of claim 44 wherein the step of exposing comprises:
exposing the plant to the acidifying agent;
waiting a period of time; and
exposing the plant to the herbicide.

46. The method of claim 1 wherein the step of manipulating comprises applying

an effective amount of a chelating agent.

47. The method of claim 46 wherein the chelating agent is selected from the group consisting of murexide, dimethylglyoxime, chromotroic acid, thiourea, cupron, CDTA, DTPA, NTA, substituted 1,10-phenanthrolines, cupral, 2-phenoxy-2-furoylmethane, phenoyl trifluoroacetone, triethylamine, EDTA, citric acid, EGTA, HEDTA, salicylic acid, and malic acid.

48. The method of claim 1 wherein the step of manipulating comprises applying an electric field to increase metal mobility.

49. A method of removing metal from an environment contaminated with the metal, the method comprising:

planting a plant that is a member of the family Brassicaceae in the environment;

applying an agent selected from the group consisting of chelating agents, acidifiers, and combinations thereof to the environment to increase metal availability to the plant planted therein;

waiting for a period; and

applying an herbicide to the environment to induce hyperaccumulation of metal in shoots of the plant.

50. The method of claim 49 wherein the plant is a member of the genus selected from the group consisting of *Brassica*, *Thlaspi*, *Alyssum*, and *Eruca*.

51. The method of claim 50 wherein the plant is a member of a species selected from the group consisting of *Brassica juncea*, *Brassica nigra*, *Brassica campestris*, *Brassica carinata*, *Brassica napus*, and *Brassica oleracea*.

52. The method of claim 51 wherein the plant is a *Brassica juncea* cultivar.

53. In a method of removing metal from an environment by cultivating a plant therein, the improvement that comprises:

exposing the plant to an inducing agent under conditions and for a time sufficient to induce the plant to hyperaccumulate metal in its shoots to a level higher than the plant would accumulate in the absence of the inducing agent.

54. The method of claim 53 wherein the inducing agent is selected from the group consisting of chelating agents, soil acidifiers, and herbicides.

55. The method of claim 53 wherein the plant is a *Brassica juncea* cultivar, the metal is lead, and the inducing agent is selected from the group consisting of at least 0.2 mM EDTA, pH less than about 3.5, and an herbicide selected from the group consisting of glyphosate, 2,4-D, and combinations thereof.

56. The method of claim 53 wherein the metal is selected from the group consisting of cadmium, copper, nickel, lead, and zinc.

57. The method of claim 56 wherein the inducing agent is selected from the group consisting of a chelator, an herbicide, and combinations thereof.

Periodic Table of the Elements

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1	H	Hydrogen [1.008]	Li	Li	Be	Be	B	C	N	O	F	Ne	He	He	He	He	He	He		
2	Li	Lithium [6.941]	Be	Boron [10.811]	Ca	Sodium [22.98977]	Mg	Magnesium [24.3050]	Al	Aluminum [26.98153]	Si	Silicon [28.0855]	P	Phosphorus [30.97376]	S	Sulfur [32.0656]	Cl	Chlorine [35.4532]	Ar	Argon [39.948]
3	Na	Na	Mg	Mg	Al	Al	Si	Si	P	P	S	S	Cl	Cl	Ar	Ar	Ar	Ar		
4	K	K	Ca	Ca	Si	Si	Al	Al	Si	Si	Si	Si	Cl	Cl	Cl	Cl	Cl	Cl		
5	Rb	Rb	Sr	Sr	Al	Al	Si	Si	Al	Al	Al	Al	Cl	Cl	Cl	Cl	Cl	Cl		
6	Cs	Cs	Ba	Ba	Al	Al	Si	Si	Al	Al	Al	Al	Cl	Cl	Cl	Cl	Cl	Cl		
7	Fr	Fr	Ra	Ra	Al	Al	Si	Si	Al	Al	Al	Al	Cl	Cl	Cl	Cl	Cl	Cl		
8	87	88	89 to 103	89 to 103	Dp	Dp	Sg	Sg	Bh	Bh	Hs	Hs	Mt	Mt	Uuu	Uuu	Uub	Uub		
9	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107		
10	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115		
11	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117		
12	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119		
13	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121		
14	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123		
15	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125		
16	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127		
17	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129		
18	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131		

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L12: Entry 6 of 6

File: USPT

DOCUMENT-IDENTIFIER: US 5711784 A

TITLE: Method for phytomining of nickel, cobalt and other metals from soil

US PATENT NO. (1) :5711784Abstract Text (1) :

Nickel/cobalt, as well as platinum and palladium metal family members are recovered from soil by growing Brassicaceae plants, specifically Alyssum in soil containing nickel/cobalt as well as other metals. The soil is conditioned by maintaining a low pH, low calcium concentration, and the addition of ammonium fertilizer and chelating agents thereto. Nickel accumulation on the order of 2.5 percent or better in above-ground tissues is achieved, which permits recovery of the metal by harvesting the above-ground plant materials, drying, and then combusting the same, to oxidize or vaporize organic materials and recover the metals sequestered therein at 10-20 fold higher concentrations than in the soil, in a form which can be used in conventional Ni refinery or smelting operations.

Brief Summary Text (12) :

The identified metal species are accumulated by growing the Alyssum in nickel-rich soil, under specific soil conditions. The conditions include: 1) lowering the soil pH, which increases the phytoavailability of nickel; 2) maintaining low Ca or lowering Ca in the soil by leaching calcium from the soil by appropriate treatments and by use of low Ca, Mg-rich soil amendments; 3) using ammonium containing or ammonium-generating nitrogen fertilizers to improve plant growth and to increase Ni hyperaccumulation due to rhizosphere acidification; and 4) applying chelating agents to the soil to improve nickel uptake by the roots of the hyperaccumulating Alyssum species. Examples of suitable chelating agents include nitrilotriacetic acid (NTA). Other chelating agents commonly used in connection with increasing soil metal mobility for plant uptake include ethylenediaminetetraacetic acid, and ethylene glycol-bis-(.beta.-aminoethylether)-N, N-tetraacetic acid. Maintenance of these four soil-conditioning factors will improve nickel hyperaccumulation in Alyssum, in excess of a 2.5 percent concentration in above-ground portions of the plant, particularly leaves and stems, which make for easy cultivation and metal recovery. This is preferable to concentration in the roots, discussed in Raskin et al, which may be an aid in soil remediation if non-leachable therefrom, but does not offer convenience for phytomining.

Detailed Description Text (5) :

These include soil pH, low calcium concentrations, use of ammonium containing or generating fertilizer rather than other N-fertilizers and application of chelating agents. Each of these is considered in turn below.

Detailed Description Text (6) :Soil pHDetailed Description Text (7) :

The maintenance of preferred pH ranges in soil is well known in agriculture for a variety of reasons. Typically, pH of soil is altered or modified so as to maintain it within a near neutral range of about 6.0-7.5. Thus, soil near a limestone foundation or other building may be treated with acidifying soil amendments so as to reduce an alkaline pH. Soil with a naturally low pH may instead be treated with limestone or similar amendment, so as to increase the soil pH. A reduced pH increases the phytoavailability of nickel and cobalt. A reduced pH increases solubility, and

optimizes the release of these metals for absorption by the roots, and translocation to the above-ground tissues of the plant. Soil pH can be maintained in any of a variety of established methods, and the methods themselves do not constitute an aspect of this invention. Preferably, soil pH is managed at a low value by addition of sulfur and use of ammonium - N fertilizers. The Alyssum species, and indeed, any plant species, grows best at its evolved optimum pH conditions. Thus, pH cannot be reduced so low as to substantially retard or inhibit plant growth. An optimum pH range for phytomining using Alyssum is a pH of 4.5 to 6.2, preferably 5.2-6.2. After extraction of economically phytominable Ni and Co from the soil, limestone application can raise soil to pH levels required by more traditional farm crops.

CLAIMS:

1. A method of recovering nickel from soil, comprising cultivating alyssum plants in soil containing nickel under conditions sufficient to permit said alyssum to accumulate nickel from the soil in above-ground tissues of said alyssum such that at least 2.5% of the air-dried above-ground tissue of said alyssum is nickel, harvesting said alyssum as biomass materials after accumulation of nickel from the soil, and recovering nickel from said biomass materials, wherein said soil is conditioned by maintaining pH of the soil at a range of 4.5 to 6.2, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration and managing said exchangeable calcium concentration at a value lower than 20% of the exchangeable Mg concentration, adding ammonium-containing fertilizer to said soil and adding chelating agents to said soil.
3. The method of claim 1, wherein said soil is conditioned by maintaining pH of the soil at a range of 4.5 to 6.2, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration and said exchangeable calcium concentration is maintained at a value lower than 20% of the exchangeable Mg concentration.

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L12: Entry 6 of 6

File: USPT

Jan 27, 1998

US-PAT-NO: 5711784

DOCUMENT-IDENTIFIER: US 5711784 A

TITLE: Method for phytomining of nickel, cobalt and other metals from soil

DATE-ISSUED: January 27, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Baker; Alan J. M.	Sheffield			GB
Li; Yin-Ming	Potomac	MD		

ASSIGNEE-INFORMATION:

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APPL-NO: 08/ 470440 [PALM]

DATE FILED: June 6, 1995

INT-CL: [06] C22 B 3/18

US-CL-ISSUED: 75/712; 47/58, 210/602

US-CL-CURRENT: 75/712; 210/602, 47/58.1R

FIELD-OF-SEARCH: 75/430, 75/710, 75/712, 210/602, 47/58

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

 [Search Selected](#) [Search ALL](#)

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> <u>5320663</u>	June 1994	Cunningham	75/432
<input type="checkbox"/> <u>5364451</u>	November 1994	Raskin et al.	

OTHER PUBLICATIONS

Salt et al., "Phytoremediation: A Novel Strategy for the Removal of Toxic Metals from the Environment Using Plants", Biotechnology, vol. 13, May 15, 1995 pp. 468-474.

ART-UNIT: 134

PRIMARY-EXAMINER: Andrews; Melvyn

ABSTRACT:

Nickel/cobalt, as well as platinum and palladium metal family members are recovered from soil by growing Brassicaceae plants, specifically Alyssum in soil containing nickel/cobalt as well as other metals. The soil is conditioned by maintaining a low pH, low calcium concentration, and the addition of ammonium fertilizer and chelating agents thereto. Nickel accumulation on the order of 2.5 percent or better in above-ground tissues is achieved, which permits recovery of the metal by harvesting the above-ground plant materials, drying, and then combusting the same, to oxidize or vaporize organic materials and recover the metals sequestered therein at 10-20 fold higher concentrations than in the soil, in a form which can be used in conventional Ni refinery or smelting operations.

5 Claims, 0 Drawing figures

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L12: Entry 6 of 6

File: USPT

US-PAT-NO: 5711784
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TITLE: Method for phytomining of nickel, cobalt and other metals from soil

DATE-ISSUED: January 27, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chaney; Rufus L.	Beltsville	MD		
Angle; Jay Scott	Elliot City	MD		
Baker; Alan J. M.	Sheffield			GB
Li; Yin-Ming	Potomac	MD		

US-CL-CURRENT: 75/712; 210/602, 47/58.1R

CLAIMS:

What is claimed is:

1. A method of recovering nickel from soil, comprising cultivating alyssum plants in soil containing nickel under conditions sufficient to permit said alyssum to accumulate nickel from the soil in above-ground tissues of said alyssum such that at least 2.5% of the air-dried above-ground tissue of said alyssum is nickel, harvesting said alyssum as biomass materials after accumulation of nickel from the soil, and recovering nickel from said biomass materials, wherein said soil is conditioned by maintaining pH of the soil at a range of 4.5 to 6.2, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration and managing said exchangeable calcium concentration at a value lower than 20% of the exchangeable Mg concentration, adding ammonium-containing fertilizer to said soil and adding chelating agents to said soil.
2. The method of claim 1, wherein said metal is recovered by drying and combusting, said harvested biomass materials, to oxidize and vaporize organic materials present.
3. The method of claim 1, wherein said soil is conditioned by maintaining pH of the soil at a range of 4.5 to 6.2, wherein said soil has an exchangeable calcium concentration and an exchangeable Mg concentration and said exchangeable calcium concentration is maintained at a value lower than 20% of the exchangeable Mg concentration.
4. The method of claim 1, wherein said Alyssum plants are selected from the group consisting of *A. murale*, *A. pintodasilvae*, *A. malacitimum*, *A. lesbiacum*, *A. fallacinum*, *A. argentum*, *A. bertolonii*, *A. tenium*, *A. heldreichii*, and mixtures thereof.
5. The method of claim 4, wherein said plants are selected from the group consisting *A. murale*, *A. pintodasilvae*, *A. malacitimum*, *A. lesbiacum*, *A.*

tenium, A. fallacinum and mixtures thereof.